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Effects of cattle and rabbit grazing on clonal expansion of spiny shrubs in wood-pastures

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Abstract

Spiny shrubs protect non-defended plants against herbivores. Therefore, they play a role for the diversity in grazed ecosystems. While the importance of these keystone nurse shrubs is presently recognized, little is known about the factors controlling them. This knowledge is required to understand the functioning of grazed ecosystems and for sustainable management.

We studied effects of cattle and rabbits on clonal expansion of *Prunus spinosa* in two ancient wood-pastures in the Netherlands. At each site we set up five blocks in grassland perpendicular to the edges of mature *Prunus* thickets, each block containing three herbivore treatments: (1) open-to-cattle-and-rabbits, (2) open to rabbits, cattle excluded, (3) cattle and rabbits excluded. We monitored the number and volume of *Prunus* ramets from 1998 to 2000 and again in 2003, 3 years after exclosure-removal to restore grazing.

For 1998–2000 ramet volume, but not ramet number, differed between treatments. Ramet volume was highest when both cattle and rabbits were excluded. Ramet volume did not differ between grazing by rabbits or cattle and rabbits combined, indicating that rabbits alone may be as effective in inhibiting clonal expansion as cattle and rabbits combined. Three years after exclosure-removal ramet number and volume had increased in all treatments. Number of ramets remained unaffected by (former) treatments. Ramet volume remained highest in the former cattle-plus-rabbits exclusion treatment, differing significantly from the ‘open-to-cattle-and-rabbits’ treatment. So, once successfully established during herbivore absence, further expansion is not prevented by cattle and rabbit grazing.

This study shows that vertebrate herbivory controls the keystone nurse-shrub in wood-pastures: combined cattle and rabbit grazing, and notably rabbits alone, inhibit expansion. Temporary herbivore absence allows expansion of ramets, which persists after herbivore-reappearance. Sustainable management of wood-pastures should allow spatial-temporal fluctuations of herbivore densities, leading to increased vegetation structure and associated biodiversity.

Zusammenfassung

Dornensträucher schützen Pflanzen ohne eigene Verteidigung vor Herbivoren. Deshalb spielen sie eine Schlüsselrolle für die Diversität in beweideten Ökosystemen. Während die Bedeutung dieser ‘Ammensträucher’ bereits anerkannt ist, ist wenig über

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die Faktoren bekannt, von denen sie kontrolliert werden. Diese Kenntnisse sind erforderlich, um die Funktionsweise beweideter Ökosysteme zu verstehen, sowie für ein nachhaltiges Management.

Wir untersuchten den Einfluss von Rindern und Kaninchen auf die klonale Ausbreitung von *Prunus spinosa* in zwei alten Waldweiden in den Niederlanden. In jedem Untersuchungsgebiet richteten wir im Grasland fünf Blöcke an den Rändern reifer *Prunus*-Dickichte ein, wobei jeder Block drei Herbivoren-Behandlungen enthielt: (1) zugänglich für Rinder und Kaninchen, (2) zugänglich nur für Kaninchen, Ausschluss von Rindern, (3) Rinder und Kaninchen ausgeschlossen. Wir protokollierten die Anzahl und das Volumen der *Prunus*-Wurzelsprosse von 1998 bis 2000 und dann wieder in 2003, d.h., drei Jahre nachdem der Herbivorenausschluss aufgehoben worden war.

Für 1998–2000 differierte das Spross-Volumen, nicht aber die Anzahl der Sprosse, zwischen den Behandlungen. Das Spross-Volumen war am höchsten, wenn sowohl Rinder als auch Kaninchen ausgeschlossen waren. Das Spross-Volumen differierte nicht zwischen Beweidung durch Kaninchen und Beweidung durch Rinder und Kaninchen, womit deutlich wird, dass Kaninchen allein die klonale Ausbreitung ebenso effektiv behindern können wie Rinder und Kaninchen zusammen. Drei Jahre nach Aufhebung des Ausschlusses hatten das Spross-Volumen und die Sprosszahl in allen Behandlungen zugenommen. Die Sprosszahl blieb durch die (frühere) Behandlung unbeeinflusst. Das Spross-Volumen blieb auf den früheren Herbivorenausschluss-Flächen am höchsten und war signifikant höher als auf den Flächen, die beiden Herbivoren zugänglich gewesen waren. Nach erfolgreicher Etablierung konnte somit die weitere Entfaltung durch Rinder- und Kaninchen-Beweidung nicht verhindert werden.

Unsere Untersuchung zeigt, dass Fraß durch Wirbeltiere eine Schlüssel-Ammenart in Waldweiden kontrolliert: Rinder und Kaninchen zusammen und insbesondere Kaninchen allein unterdrücken die Entfaltung. Das zeitweise Fehlen von Herbivoren erlaubt die Entfaltung der Sprosse, die nach der Rückkehr der Herbivoren bestehen bleiben. Ein nachhaltiges Management von Waldweiden sollte raum-zeitliche Schwankungen der Herbivorendichten zulassen, was zu vermehrter Vegetationsstruktur und Biodiversität führt.

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Keywords: Herbivory; Browsing; Exclosure; Plant–herbivore interaction; Shrub encroachment; Pasture woodland; Nurse shrub; Clonal reproduction

Introduction

Grazing is thought to increase the temporal and spatial heterogeneity in vegetation structure – with mosaics of short grassland, tall swards, shrubs and trees shifting over time and space – thus supporting a rich biodiversity (Olf et al. 1999). Various studies on ancient grazed ecosystems indeed suggest that large herbivores create and maintain this landscape heterogeneity and inherent diversity (Vera 2000; Bakker, Olf, Vandenbergh, De Maeyer, & Smit 2004; Smit, Beguin, Buttler & Müller-Schärer 2005; Smit, den Ouden & Müller-Schärer 2006). Also on abandoned agricultural land, heathlands or riverine floodplains ('nature development projects') the introduction of large herbivores leads to heterogeneous landscapes (Bokdam & Gleichman 2000; Van Uytvanck, Maes, Vandenhoute & Hoffmann 2008).

Plants with defence mechanisms against large herbivores – physically via spines and thorns or chemically via alkaloids or high lignin/cellulose content – play a crucial role for the heterogeneity and diversity in these grazed ecosystems. They protect non-defended species against large herbivores and facilitate their establishment, survival, flowering and seed set, thereby increasing the overall species richness of the community (Rousset & Lepart 1999; Milchunas & Noy-Meir 2002). These 'nurse' plants also protect palatable tree seedlings against large herbivores and thereby initiate the establishment of isolated forest patches in the landscape (Rousset & Lepart 1999; Bakker et al. 2004; Smit et al. 2005, 2006; Van Uytvanck et al. 2008) in accordance with the shifting mosaic

theory (Olf et al. 1999). However, while the importance of nurse plants in grazed ecosystems is now well recognized (Smit, Rietkerk, & Wassen 2009), little is known about the factors driving the dynamics of nurse plants. Such knowledge is of crucial importance for a better understanding of the functioning of both ancient and newly created grazed ecosystems and their sustainable conservation management. In this study we test the impact of vertebrate herbivores on the clonal expansion of the spiny nurse shrub *Prunus spinosa* (Blackthorn), which plays a keystone role for establishment of palatable *Quercus robur* (Pedunculate oak) in ancient wood-pasture ecosystems in Western-Europe (Olf et al. 1999; Bakker et al. 2004).

Grazed ecosystems generally contain multiple species of vertebrate herbivores which may differ in feeding selectivity. While large bulk feeders (e.g. cattle, African buffalo) consume high quantities of low quality material (high C:N ratio), small selective feeders (e.g. roe deer, dikdik) generally include more browse in their diet than large bulk feeders (Hofmann 1989) and may therefore be important regulators of shrub encroachment in temperate grasslands (Weltzin, Archer, & Heitschmidt 1997). Correlative studies indeed show that shrub expansion over decades coincides with the reduction or disappearance of prairie dogs and rabbit densities in respectively prairies (Weltzin et al. 1997) and wood-pastures (Bakker et al. 2004). Van der Wal, van Wieren, Beucher, and Bos (2000) showed that browsing by European hares can delay shrub encroachment on salt marshes by more than 25 years. Older observa-

tional studies, with a more anecdotic character, report an increase of *Ulex europaeus*, *Rosa spinosissima*, *Crataegus monogyna*, *Juniperus communis* and *Rubus* spp. since the advent of myxomatosis in chalk-grasslands in the UK in 1954 (Thomas 1960), similarly suggesting regulatory effects by a small selective feeder. However, more properly controlled experiments testing the impact of the different herbivores on shrub expansion in temperate grasslands are very scarce (see Weltzin et al. 1997).

The potential inhibiting effects of herbivores on shrubs may be highest at the young stage when defence mechanisms such as spines and thorns have not yet fully developed (Rackham 1980), but once established, effects on ramets may disappear. Therefore, the expansion of nurse shrubs may mainly occur during temporary absence or low densities of controlling herbivores. These temporary low densities of herbivores may be caused by migrations, population crashes due to diseases, hunting or management practices and could form a ‘window-of-opportunity’ for clonal ramets to establish. Such fluctuations due to diseases are still commonly occurring for wild populations of smaller herbivores such as hare or rabbits (e.g. van de Bildt et al. 2006). In most West-European nature areas however, large herbivores can rarely freely migrate over large distances due to the restricted surface areas, and population crashes are rare due to strict management of herbivore populations. Temporary absence of herbivores can however simply be manipulated by exclosures.

In this study we aimed to quantify the impact of vertebrate herbivory on the expansion of a clonal nurse shrub in ancient grazed wood-pastures. We investigated the effects of a selective feeder (rabbit) and a bulk feeder (cattle) using a stepwise exclosure design, consecutively excluding cattle and both cattle and rabbits. After 3 years, all exclosures were removed in order to study the impact of return of these herbivores. We hypothesized that (1) vertebrate herbivory suppresses clonal shrub expansion; (2) the role of small selective feeders in suppressing clonal shrub expansion is relatively large; and (3) return of vertebrate herbivores after temporary absence does not avert the further growth of established ramets.

Methodology

Study sites

This study was performed at two ancient wood-pastures – Junner Koeland (52° 32'N, 6° 29'E) and Prathoek (52° 31'N, 6° 30'E), two nature reserves of 100 and 22 ha, respectively, along the river Overijsselse Vecht, The Netherlands. These areas used to be communal grazing land for farmers from nearby villages, and have probably been grazed by livestock since medieval times (Bakker et al. 2004). Both sites consist of a mosaic of short grazed grasslands (dominated by *Festuca rubra*, *Agrostis capillaris*), swards of tall unpalatable plants (dominated by *Juncus effusus*, *Urtica dioica*, *Deschampsia cespitosa*), shrub thickets (dominated by *Prunus spinosa*) and

woodland (predominantly Pedunculate oak *Quercus robur*). *Prunus spinosa* (hereafter *Prunus*), a deciduous shrub or small tree up to 5 m tall with wide-angled and stiff spiny branches, offers physical protection against large herbivores for seedlings and saplings of palatable woody species such as oak and ash (*Fraxinus excelsior*) (Bakker et al., 2004). *Prunus* mainly regenerates clonally via horizontal rhizomes that can quickly invade surrounding grassland (Coops 1988). From 1998 to 2003 both sites were grazed by cattle at an average rate of 0.4 LU (livestock units) ha⁻¹ during spring and summer. Estimated densities of European rabbits (*Oryctolagus cuniculus*) for the period 1998–2003 were also similar between both sites (10.9 and 9.5 ha⁻¹ for Junner Koeland and Prathoek, respectively). Other vertebrate herbivores that occur in the study sites are roe deer (*Capreolus capreolus*), brown hare (*Lepus europaeus*) – both at very low densities – and common vole (*Microtus arvalis*), bank vole (*Clethrionomys glareolus*) and wood mouse (*Apodemus sylvaticus*).

Experimental design

In May 1998 we selected five blocks of 10 m × 15 m in each study site, with individual blocks widely spread over the study sites (smallest distance between blocks being ca. 30 m). Each block was placed in short grassland, perpendicular to the outer edge of a separate mature *Prunus spinosa* shrub thicket, and consisted of three compartments of 4 m × 9 m, each subjected to one of three treatments: (1) CR: open-to-cattle-and-rabbits, (2) R: open to rabbits, cattle excluded by barbwire at 0.5 and 1 m height, and (3) NO: exclusion of both cattle and rabbits by 1-m high chicken-mesh (Fig. 1). Each 9 m × 4 m grazing treatment was subdivided into 36 plots of 1 m², permanently marked with wooden sticks, where we counted the number of individual *Prunus* ramets and measured the height and diameter (at base) of all individual ramets. Measurements started at June 1998 and were repeated in 1999 and 2000. After the measurements in 2000 all exclosures were removed with the

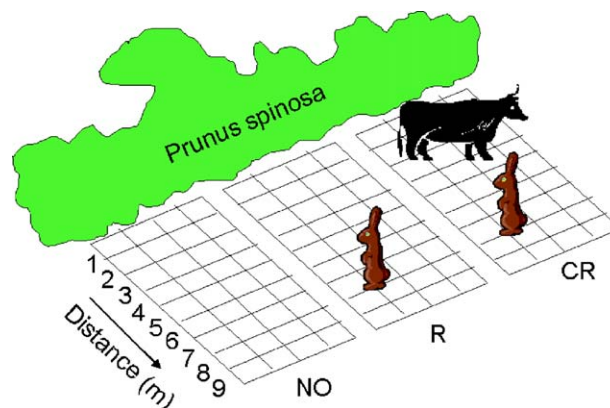


Fig. 1. Schematic overview of experimental design of one block (15 m × 10 m) in short grassland adjacent to a mature *Prunus spinosa* shrub, with plots subjected to cattle and rabbits (CR), rabbits (R) and no cattle and rabbits (NO).

aim to mimic reintroduction or recovery of both herbivore species after an absence of 3 years. All measurements on *Prunus* ramets were repeated in October 2003, 3 years after enclosure-removal.

To estimate rabbit densities in the three treatments we regularly counted and removed rabbit droppings (pellets) in one permanent 4 m² plot per treatment in each block in both sites in 1998, 1999, 2000 and 2003.

Data analysis

For each study year (1998, 1999, 2000 and 2003) we calculated individual ramet volume (v_i) using individual ramet height h_i [cm] and ramet diameter d_i [cm], according to the following formula: $v_i = \pi h_i d_i^2 / 12$ [cm³]. Hence, ramet volume was estimated assuming a cone shape. Both number and volume of ramets were then summed up over each 4 × 9 compartment (total number and volume of ramets per 36 m² compartment) and used as replicated units (5 per treatment per site) for further analyses. We used repeated-measures ANOVA to test for the effects of time (within-subject effects) and for effects of site, block (random factor), grazing and site × grazing on number and volume of ramets over the period 1998–2000 (between-subject effects). We used Greenhouse-Geisser adjustment of degrees of freedom when the assumption of sphericity was violated (unequal variances between groups). When the factor time was significant, we performed a two-way ANOVA to test for effects of site, block (random factor), grazing and site × grazing for the individual years 1998, 1999, 2000 and also for 2003 (3 years after removal of the enclosures). Tukey-tests were used for multiple comparisons between levels of significant factors.

To test whether enclosure-removal had differential effects on the increase of ramet numbers and volume in the three treatments, we calculated the relative expansion (δ) by subtracting the number and volume values for 2000 from the values for 2003, divided by the values for 2000. We used ANOVA to test for differences between site, block, grazing and site × grazing, after $\log_{10}(x+1)$ transformation of the response to satisfy the assumptions of normality.

Rabbit densities were estimated from the pellet counts according to the following formula as used by Bankert, in 't Groen and van Wieren (2003): $d = (10,000\mu)/(\rho \times T \times A)$, where d = density estimate of rabbits (rabbits ha⁻¹), μ = mean number of pellets counted, ρ = defecation rate (pellets rabbit⁻¹ day⁻¹), T = time between pellet removal and pellet counting (day) and A = surface of each sampling unit (m²). We assumed a defecation rate ρ of 400 pellets per rabbit/day (slightly higher than the 373 pellets/day found by Redondo 2009). Rabbit densities were analyzed with repeated-measures ANOVA, testing for the effects of time (within-subjects effects), and of treatment, site and treatment × site for the period 1998–2000 (between-subject effects). We used Greenhouse-Geisser adjustment of degrees of freedom when the assumption of sphericity was violated.

Significant differences between treatments were followed by a Tukey-test. Furthermore, we tested for differences between the CR and R treatments for the period 1998–2000 to find out if rabbits showed a preference for one of these treatments. For the year 2003 (after enclosure-removal) we applied a two-way ANOVA with site, grazing and site × grazing as factors and rabbit density as response. We used SPSS 16.0 for Windows (Chicago, IL, USA) for all statistical analyses.

Results

In total we measured 3735 ramets between 1998 and 2003, with on average 5.48 ± 0.09 ramets (means ± se, range: 1–55) per full 1 m² plot, with a height of 36.0 ± 0.55 cm (means ± se, range: 1–400) and a diameter of 0.40 ± 0.006 cm (means ± se, range: 0.1–5.1). At block level, number of ramets ranged from 15 to 618 and ramet volume ranged from 0.3 to 36297.9 cm³.

Repeated-measures ANOVA showed that time and time × block had significant effects on the number of ramets ($F_{1,3,25,3} = 24.878$; $p < 0.001$ and $F_{5,1,25,3} = 3.556$; $p < 0.05$), while time × site, time × grazing and time × site × grazing were not significant ($F_{1,3,25,3} = 2.658$, $F_{2,5,25,3} = 1.102$, $F_{2,5,25,3} = 0.280$). The number of ramets over time did not show a clear pattern (Fig. 2A, data shown for sites com-

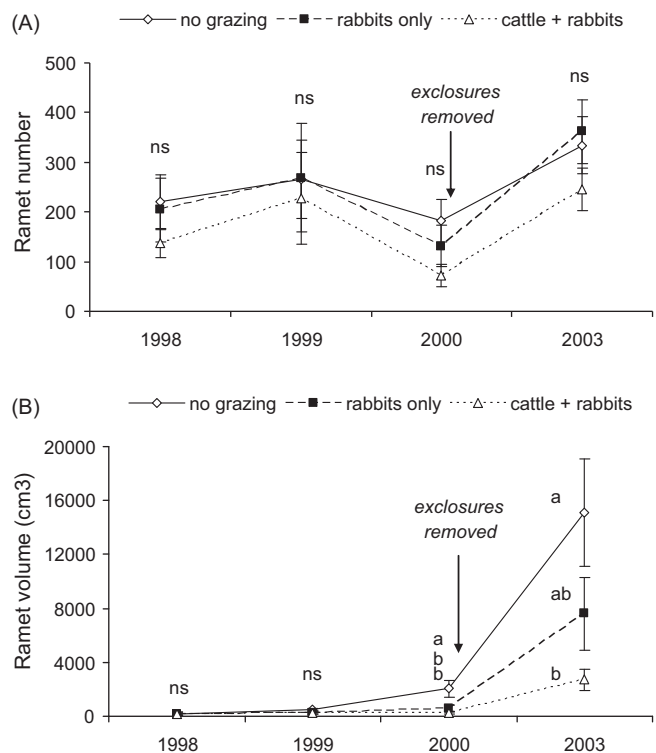


Fig. 2. Number of ramets (A) and ramet volume (B) for the years 1998, 1999, 2000 and for 2003 (3 years after removal of enclosures). Depicted values are means ± 1 se. Different letters indicate significant differences between treatments within years at $p < 0.05$.

bined as we were interested in general patterns and not in between-site variation). There were no effects of grazing-treatment or site \times grazing on number of ramets for the period 1998–2000 nor for separate years (Fig. 2A; Table 1A–D), but site was significant, with more ramets in Junner Koeland than in Prathoek.

In contrast, ramet volume showed a clear pattern over time (Fig. 2B), with a strong increase when cattle and rabbits were excluded, a weak increase when grazed by rabbits only and a negligible increase when grazed by cattle and rabbits. Repeated-measures ANOVA showed that time was significant for ramet volume ($F_{1,0,21,0} = 23.266$; $p < 0.001$) and so where most time-interactions (time \times site: $F_{1,0,21,0} = 6.854$; $p < 0.05$, time \times grazing: $F_{2,1,21,0} = 10.996$; $p < 0.001$, time \times site \times grazing: $F_{2,1,21,0} = 5.805$; $p < 0.01$) except for time \times block ($F_{1,0,21,0} = 2.186$). Furthermore – and in contrast to number of ramets – ramet volume was significantly affected by grazing, site and their interaction, while block effects were not significant (Table 1A). Overall, ramet volume was higher when cattle and rabbits were excluded (NO) than when rabbits (R) and cattle and rabbit (CR) were present (Fig. 2B), but ramet volume did not differ between the rabbit (R) and cattle and rabbit (CR) treatment. Differences in ramet volume between treatments were not significant for the first 2 years but became significant in 2000 (Table 1D), with higher values when both cattle and rabbits were excluded ($2044 \pm 613 \text{ cm}^3$) than when rabbits only ($619 \pm 249 \text{ cm}^3$), or cattle and rabbits ($267 \pm 77 \text{ cm}^3$) had access (all means \pm se given). Ramet volume was consistently higher in Junner Koeland than in Prathoek, and the effects of grazing were also stronger, as reflected by the significant grazing \times site interaction.

Exclosure-removal in 2000 led to a significant expansion of both ramet number and volume in 2003, valid for all treatments (paired t -tests 2000–2003, NO: $t = -3.318$ and $t = -3.724$; R: $t = -2.634$ and $t = -3.474$; CR: $t = -3.237$ and $t = -3.969$ for ramet number and ramet volume respectively; $p < 0.05$). Results of the ANOVA for 2003 showed that ramet numbers were again unaffected by the former grazing-treatments (Table 1E). Only effects of site were found, with significantly higher numbers for Junner Koeland than Prathoek. In contrast, ramet volume differed between former grazing-treatments, while site and site \times grazing were no longer significant. Differences between former treatments for 2003 were only significant between the NO and CR treatment (Fig. 2B).

Yet, the relative increase (δ) of volume after exclosure-removal was not affected by site ($F_{1,20} = 0.000$), block ($F_{4,20} = 1.593$), grazing ($F_{2,20} = 0.358$) or site \times grazing ($F_{2,20} = 0.555$), showing similar values for the grazing-treatments after exclosure-removal (all increased). The relative increase (δ) of number of ramets was not affected by site ($F_{1,20} = 0.605$), grazing ($F_{2,20} = 0.066$) or site \times grazing ($F_{2,20} = 0.134$), but was affected by block ($F_{4,20} = 0.328$; $p = 0.028$).

Table 1. Results of repeated-measures ANOVA on number of ramets and ramet volume (cm^3) per 36-m^2 compartment over the period 1998–2000 (A), and ANOVA results for individual years with exclosure (1998, 1999, 2000) and 3 years after removal of fences (2003) (B–E).

Response	Source of variation	Df	(A) 1998–2000			(B) 1998			(C) 1999			(D) 2000			(E) 2003		
			MS	F		MS	F		MS	F		MS	F		MS	F	
Number of ramets	Site (S)	1	180275.4	7.186*		71150.7	9.849**		101152.1	5.284*		22687.5	5.333*		133466.7	11.188**	
	Block	4	49666.7	1.980		9388.7	1.300		55246.5	2.886*		4717.4	1.109		7901.6	0.662	
	Grazing (G)	2	20207.1	0.805		7371.7	1.020		8138.7	0.425		10796.8	2.538		17574.9	1.473	
	S \times G	2	12997.1	0.518		3573.3	0.495		4831.4	0.252		6144.4	1.444		4370.8	0.366	
Ramet volume	Error	20	25087.9			7224.5			19144.8			4254.4			11929.0		
	Site (S)	1	3811754.8	6.851*		30315.2	0.515		396206.0	4.169		6646316.6	7.568*		9753.2	0.000	
	Block	4	487847.3	0.877		75785.6	1.286		54259.1	0.571		1397923.6	1.592		6553377.5	0.762	
	Grazing (G)	2	3821917.7	6.869**		3014.7	0.051		199532.7	2.100		8851619.4	10.079**		391308463.3	4.553*	
	S \times G	2	2623603.7	4.716*		97939.7	1.663		238788.0	2.513		5049151.9	5.749*		85500345.7	0.995	
	Error	20	556376.4			58910.0			95034.7			878247.6			85951454.4		

Significant values are indicated in bold.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

Table 2. Rabbit densities (no. individuals ha⁻¹) averaged over the two study sites and grazing-treatments (NO: no rabbits and cattle, R: only rabbits, CR: rabbits and cattle) for the period 1998–2000, for individual years with exclosures (1998, 1999, 2000) and three years after removal of fences (2003). Years with significant differences in rabbit densities are marked by an asterisk and differences between grazing-treatments (within years) are marked with different superscript letters ($p < 0.05$; Tukey-test).

Grazing	1998–2000*	1998*	1999	2000*	2003
NO	0 ^a	0 ^a	0	0 ^a	1.90 ± 2.24
R	10.25 ± 6.85 ^b	21.31 ± 15.40 ^b	5.58 ± 8.29	3.85 ± 4.55 ^b	3.81 ± 4.14
CR	14.98 ± 16.87 ^b	23.17 ± 17.18 ^b	17.41 ± 31.84	4.37 ± 5.56 ^b	2.47 ± 3.60

Rabbit densities showed an overall decrease from 1998 to 2003 (Table 2), most likely due to the effects of the rabbit hemorrhagic disease. Repeated-measures ANOVA over the period 1998–2000 (exclosure experiment) showed no effects of site ($F_{1,20} = 0.032$), block ($F_{4,20} = 1.148$) or site × grazing ($F_{2,20} = 0.266$), but rabbit densities differed significantly between treatments ($F_{2,20} = 4.945$; $p = 0.018$), obviously due to exclusion of rabbits in the NO treatment (Table 2). Rabbit densities did not differ between the CR and R treatments ($F_{1,20} = 0.615$; $p = 0.444$), indicating that rabbits showed no preference for either CR or R (Table 2). After exclosure-removal, rabbit densities in 2003 were not affected by any of the factors site ($F_{1,20} = 3.527$), block ($F_{4,20} = 1.676$), grazing ($F_{2,20} = 0.981$) or site × grazing ($F_{2,20} = 1.027$).

Discussion

Results of this study show that grazing by cattle and rabbits exert strong inhibiting effects on *Prunus* expansion into adjacent grassland in the studied ancient wood-pastures. Ramet volume, but not number of ramets, was heavily affected: 3 years of herbivore-exclusion led to a large increase of ramet volume, while grazing suppressed this expansion significantly. These findings are in accordance with our first hypothesis that vertebrate herbivory suppresses clonal shrub expansion.

Combined grazing by cattle and rabbits (CR) had the largest impact on the clonal expansion. After 3 years, this treatment led to a 7.6-fold reduction in ramet volume compared with the ungrazed control (NO), while rabbit grazing (R) led to a 3.3-fold reduction compared with NO. However, ramet volume did not differ between CR and R over the entire period 1998–2000, nor for any year separately (Fig. 2B). This implies that the impact of rabbits in inhibiting shrub expansion is relatively large, which is in agreement with our second hypothesis. Impact of rabbits on shrub expansion was already proposed by Bakker et al. (2004) who showed that clonal expansion of *Prunus* was negatively correlated with rabbit abundance, but our study is the first that supports this idea with a controlled experiment. Similar effects of small-sized selective feeders were found by Augustine and McNaughton (2004) who showed that particularly browsing by the small-sized selective dik-dik antelope (*Madoqua kirkii*) – and not the non-selective feeding by cattle or elephants – reduced *Aca-*

cia expansion in East African savannas. The relative strong impact of rabbits on shrub expansion can firstly be explained by their diet, which partly consists of woody material (varying from 0 to 40% depending on habitat and season) and particularly of the green parts such as leaves, young ramets and shoots that are selected for in winter, but also during summer (Kaetzk, Niedermeier & Masseti 2003). Secondly, burrows of rabbits are preferably situated underneath protective structures such as shrubs, offering protection against birds of prey and larger mammalian predators (Bakker et al. 2005; Van Uytvanck et al. 2008). As most rabbit foraging takes place within 10 m from burrows (Kolb 1991; Dekker 2007), it is likely that nearby *Prunus* shrubs suffer from intense rabbit browsing.

The strong reduction in ramet volume at CR compared with NO indicates that also cattle contributed to the suppression of *Prunus* expansion. Although considered non-selective bulk feeders, cattle do browse upon green leaves and (less thorny) twigs of *Prunus spinosa* (Buttenschon & Buttenschon 1978; Coops 1988). Young and small ramets sprouting in grasslands may also be grazed by chance together with more tasty surrounding focal species (Smit et al. 2006). This effect is probably weaker for older and taller ramets because they are more obvious to large herbivores (Rao, Iason, Hulbert, Elston & Racey 2003).

The somewhat stronger effect of rabbits and cattle together could also be the result of facilitation. Large bulk feeders can facilitate feeding of small selective herbivores (Arsenault & Owen-Smith 2002). Facilitation of cattle for rabbits has been found in Junner Koeland's grasslands (Bakker, Olff & Gleichman 2009) where rabbits preferred cattle-grazed fields, probably due to the short nutritious vegetation that also supplies good outlook possibilities and escape routes from predators. However, cattle did not facilitate rabbits nearby shrubs in our study as there were no differences in rabbit densities between the CR and R treatments. On the contrary, as rabbits strongly inhibit shrub expansion, they may eventually facilitate cattle feeding by maintaining grassland open. This is also suggested by Weltzin, Archer, and Heitschmidt (1997) who describe that prairie dogs may prevent tree establishment and enhance prairie-openness with positive results for cattle. This may be a more general phenomenon, but very little is known about this type of facilitation.

We acknowledge that we cannot assess the impact of cattle grazing alone (without rabbits) on shrub expansion with

our study design. We choose for a stepwise exclosure design for reasons of feasibility. A treatment allowing cattle to enter while excluding rabbits would however be ideal for disentangling the individual effects of both herbivore types. Yet, based on our results we can conclude that combined cattle and rabbit grazing importantly suppresses *Prunus* expansion in wood-pastures, and that particularly the role of rabbits is relatively large. Our results underline the generally overlooked potential for small selective herbivores to control the dynamics of woody vegetation in grazed ecosystems (Weltzin et al. 1997). By controlling the expansion of spiny shrubs – and the dependent recruitment of palatable trees within these shrubs – rabbits play a similar key engineering role in the landscape as is regularly claimed for large herbivores (Olff et al. 1999; Bokdam & Gleichman 2000).

Three years after exclosure-removal both ramet number and volume had increased by a factor 2.4 and 8.5, respectively. So, re-established grazing of both herbivores could not set-back the established *Prunus* expansion. These results are in agreement with our third hypothesis. Absence of cattle and rabbits offers a ‘window-of-opportunity’ for establishment of young, unprotected ramets. After 2–3 years, when more woody tissue and defensive thorns have developed, they become less attractive and less sensitive to herbivory. Also, as suggested by Coops (1988), shrub expansion above a certain height can no longer be inhibited by grazers. Obviously, this ‘browse-line’ is reached earlier by rabbits (ca. 1 m) than by cattle (ca. 1.5 m). Above this browse-line volume increase of *Prunus* is probably no longer limited by vertebrate herbivory. Indeed, the maximum ramet heights per 1 m² in the NO treatments were approaching this browse-line for rabbits already in 2000 (87.5 ± 2.9 cm; mean \pm se) and continued to reach the cattle browse-line in 2003 (127.5 ± 4.9 cm), while the R and CR treatments reached maximum ramet heights of 90.4 ± 4.7 cm and 59.8 ± 3.2 cm in 2003, respectively.

In contrast with ramet volume, ramet numbers were not affected by the grazing-treatments during the entire study period. This indicates that, once established, ramets can rapidly expand in height and width (increasing ramet volume) depending on the grazing regime, while shrub expansion via establishment of new ramets is fairly limited in all grazing regimes. This has probably to do with the ramet production of the parent shrubs that is not directly influenced by the different grazing-treatments; grazing by rabbits and cattle can, of course, become effective after the ramets sprout from the rhizome network in the soil. These sprouting ramets may be grazed when emerging in grassland and grazers are present, but will rapidly expand when grazing ceases.

The fact that shrub expansion increased during the last 3 years after exclosure-removal (Fig. 2) may be explained by the declining rabbit population, again supporting the idea that particularly rabbits play a crucial role. Our results are in line with observations of apparent cohorts of *Prunus* recruitment in shrub thickets that were mainly due to the fluctuations of rabbit densities (Bakker et al. 2004). Therefore, we may expect that the recent decline and slow recovery of rabbit

populations due the rabbit hemorrhagic disease (van de Bildt et al. 2006) will be reflected in new recruitment cohorts of *Prunus* – comparable to the increase of woody plants after the advent of myxomatosis (Thomas 1960) – but also of associated palatable tree recruits that profit from the protection against herbivores. The population crash of rabbits will also have consequences for cattle forage and accessibility as the increased shrub volume will reduce the amount of grassland, as shown in this study.

Our study shows that once *Prunus* shrubs are present, they can rapidly expand clonally, depending on herbivore presence. The next question to be answered is how new *Prunus* shrubs establish in these grazed landscapes, hence via seeds. The abundant seed availability and seed dispersal – mainly by thrushes (Snow & Snow 1988) – do not seem to be limiting factors in wood-pastures. However, post-dispersal seed(ling) and sapling survival may be limiting, as blackthorn seeds are preferred forage for granivorous rodents (Kollmann, Coomes, & White 1998) and protective vegetation may be needed for the still poorly protected recruits. Future research should focus on the establishment phase of spiny shrubs in wood-pastures.

Currently, the management of grazed nature areas in Western-Europe focuses on large herbivores; densities and types (mostly cattle, horses, sheep or deer) are frequently manipulated in order to steer the vegetation in a ‘desired’ direction. Our study now shows that besides the acknowledged role of large herbivores, particularly small selective feeders such as rabbits play a major role in controlling vegetation dynamics. Furthermore, we show that temporary herbivore absence – due to a (mimicked) population crash or migrations – can lead to increased vegetation structure, with expected positive impact on associated biodiversity. Such fluctuations in herbivore populations presently rarely occur due to the fragmented distribution and limited size of nature areas that do not allow significant migrations, and due to the strict management of herbivore populations. We suggest that for increased dynamics, heterogeneity and diversity in grazed nature areas management should consider allowing such fluctuations in herbivore populations.

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References

- Arsenault, R., & Owen-Smith, N. (2002). Facilitation versus competition in grazing herbivore assemblages. *Oikos*, 97, 313–318.

- Augustine, D. J., & McNaughton, S. J. (2004). Regulation of shrub dynamics by native browsing ungulates on East African rangeland. *Journal of Applied Ecology*, 41, 45–58.
- Bakker, E. S., Olff, H., Vandenbergh, C., De Maeyer, K., Smit, R., Gleichman, J. M., et al. (2004). Ecological anachronisms in the recruitment of temperate light-demanding tree species in wooded pastures. *Journal of Applied Ecology*, 41, 571–582.
- Bakker, E. S., Reijnders, R., Olff, H., & Gleichman, J. (2005). Experimental manipulation of predation risk and food quality: Effect on grazing behaviour in a central-place foraging herbivore. *Oecologia*, 146, 157–167.
- Bakker, E. S., Olff, H., & Gleichman, J. M. (2009). Contrasting effects of large herbivore grazing on smaller herbivores. *Basic and Applied Ecology*, 10, 141–150.
- Bankert, D., in 't Groen, K. C. G., & Van Wieren, S. E. (2003). A review of the transect method by comparing it with three other counting methods to estimate rabbit (*Oryctolagus cuniculus*) density. *Lutra*, 46, 27–34.
- Bokdam, J., & Gleichman, M. (2000). Effects of grazing by free-ranging cattle on vegetation dynamics in continental north-west European heathland. *Journal of Applied Ecology*, 37, 415–431.
- Buttenschon, J., & Buttenschon, R. M. (1978). The effect of browsing by cattle and sheep on trees and bushes. *Natura Jutlandica*, 20, 79–94.
- Coops, H. (1988). Occurrence of blackthorn (*Prunus spinosa* L.) in the area of Mols Bjerger and the effect of cattle and sheep grazing on its growth. *Natura Jutlandica*, 22, 169–176.
- Dekker, J. J. A. (2007). *Rabbits, refuges and resources. How foraging of herbivores is affected by living in burrows*. PhD thesis. Wageningen University.
- Hofmann, R. R. (1989). Evolutionary steps of ecophysiological adaptation and diversification of ruminants: A comparative view of their digestive system. *Oecologia*, 78, 443–457.
- Kaetzke, P., Niedermeier, J., & Masseti, M. (2003). *Oryctolagus cuniculus* (Linné, 1758) – Europäisches Wildkaninchen. In J. Niethammer, & F. Krapp (Eds.), *Handbuch der Säugetiere Europas. Band 3/III Hasentiere*. Wiebelsheim: Aula-Verlag.
- Kolb, H. H. (1991). Use of burrows and movements of wild rabbits (*Oryctolagus cuniculus*) in an area of hill grazing and forestry. *Journal of Applied Ecology*, 28, 892–905.
- Kollmann, J., Coomes, D. A., & White, S. M. (1998). Consistencies in post-dispersal seed predation of temperate fleshy-fruited species among seasons, years and sites. *Functional Ecology*, 12, 683–690.
- Milchunas, D. G., & Noy-Meir, I. (2002). Grazing refuges, external avoidance of herbivory and plant diversity. *Oikos*, 99, 113–130.
- Olff, H., Vera, F. W. M., Bokdam, J., Bakker, E. S., Gleichman, J. M., Maeyer, K. d., et al. (1999). Shifting mosaics in grazed woodlands driven by the alternation of plant facilitation and competition. *Plant Biology*, 1, 127–137.
- Rackham, O. (1980). *Ancient woodland. Its history, vegetation and uses in England*. London, UK: Edward Arnold Publishers Ltd.
- Rao, S. J., Iason, G. R., Hulbert, I. A. R., Elston, D. A., & Racey, P. A. (2003). The effect of sapling density, heather height and season on browsing by mountain hares on birch. *Journal of Applied Ecology*, 40, 626–638.
- Redondo, P. G. (2009). Number of faecal pellets dropped daily by the wild rabbit (*Oryctolagus cuniculus*). *Journal of Animal and Veterinary Advances*, 8, 2635–2637.
- Rousset, O., & Lepart, J. (1999). Shrub facilitation of *Quercus humilis* regeneration in succession on calcareous grasslands. *Journal of Vegetation Science*, 10, 493–502.
- Smit, C., Béguin, D., Buttler, A., & Müller-Schärer, H. (2005). Safe sites for tree regeneration in wooded pastures: A case of associational resistance? *Journal of Vegetation Science*, 16, 209–214.
- Smit, C., Den Ouden, J., & Müller-Schärer, H. (2006). Unpalatable plants facilitate tree sapling survival in wooded pastures. *Journal of Applied Ecology*, 43, 305–312.
- Smit, C., Rietkerk, M., & Wassen, M. J. (2009). Inclusion of biotic stress (consumer pressure) alters predictions from the stress gradient hypothesis. *Journal of Ecology*, 97, 1215–1219.
- Snow, B., & Snow, D. (1988). In J. Niethammer, & F. Krapp (Eds.), *Birds and berries. A study of an ecological interaction. T and AD Poyser, Calton*.
- Thomas, A. S. (1960). Changes in vegetation since the advent of myxomatosis. *Journal of Ecology*, 48, 287–305.
- van de Bildt, M. W. G., van Bolhuis, G. H., van Zijderveld, F., van Riel, D., Drees, J. M., Osterhaus, A. D. M. E., et al. (2006). Confirmation and phylogenetic analysis of Rabbit Hemorrhagic Disease Virus in free-living rabbits from the Netherlands. *Journal of Wildlife Disease*, 42, 808–812.
- van der Wall, R., van Wijnen, H., van Wieren, S., Beucher, O., & Bos, D. (2000). On facilitation between herbivores: How Brent Geese profit from brown hares. *Ecology*, 81, 969–980.
- Van Uytvanck, J., Maes, D., Vandenhaute, D., & Hoffmann, M. (2008). Restoration of woodpasture on former agricultural land: The importance of safe sites and time gaps before grazing for tree seedlings. *Biological Conservation*, 141, 78–88.
- Vera, F. W. M. (2000). *Grazing ecology and forest history*. Oxon: CAB International.
- Weltzin, J. F., Archer, S., & Heitschmidt, R. K. (1997). Small-mammal regulation of vegetation structure in a temperate Savanna. *Ecology*, 78, 751–763.